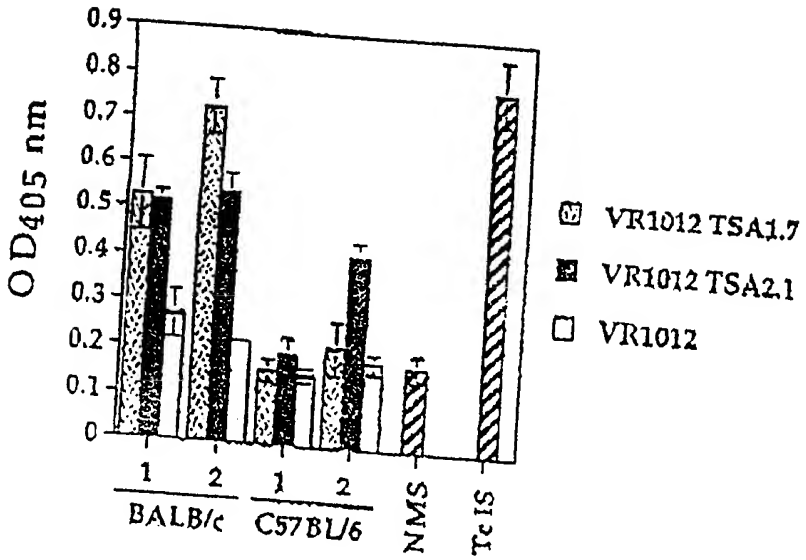

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD405 nm
1
2
1
2
BALB/c
C57BL/6
NMS
Tc IS
VR1012 TSA1.7
VR1012 TSA2.1
VR1012

Fig. 1

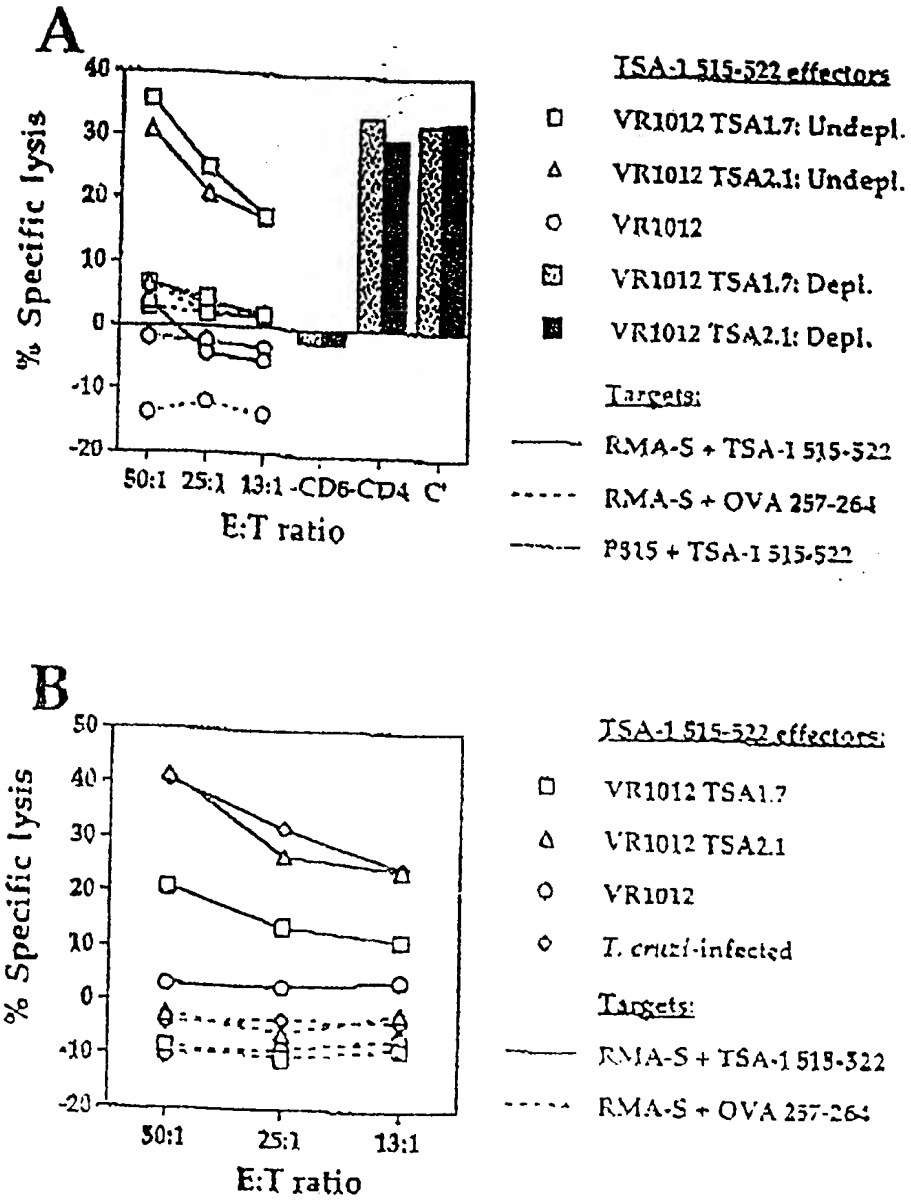

A
% Specific lysis
40
30
20
10
0
-10
-20
50:1 25:1 13:1 -CD8-CD4 C'
E:T ratio
TSA-1 515-522 effectors
VR1012 TSA1.7: Undepl.
VR1012 TSA2.1: Undepl.
VR1012
VR1012 TSA1.7: Depl.
VR1012 TSA2.1: Depl.
Targets:
RMA-S + TSA-1 515-522
RMA-S + OVA 257-264
P815 + TSA-1 515-522
B
% Specific lysis
50
40
30
20
10
0
-10
-20
50:1
25:1
13:1
E:T ratio
TSA-1 515-522 effectors:
VR1012 TSA1.7
VR1012 TSA2.1
VR1012
T. cruzi-infected
Targets:
RMA-S + TSA-1 515-522
RMA-S + OVA 257-264

Fig. 2

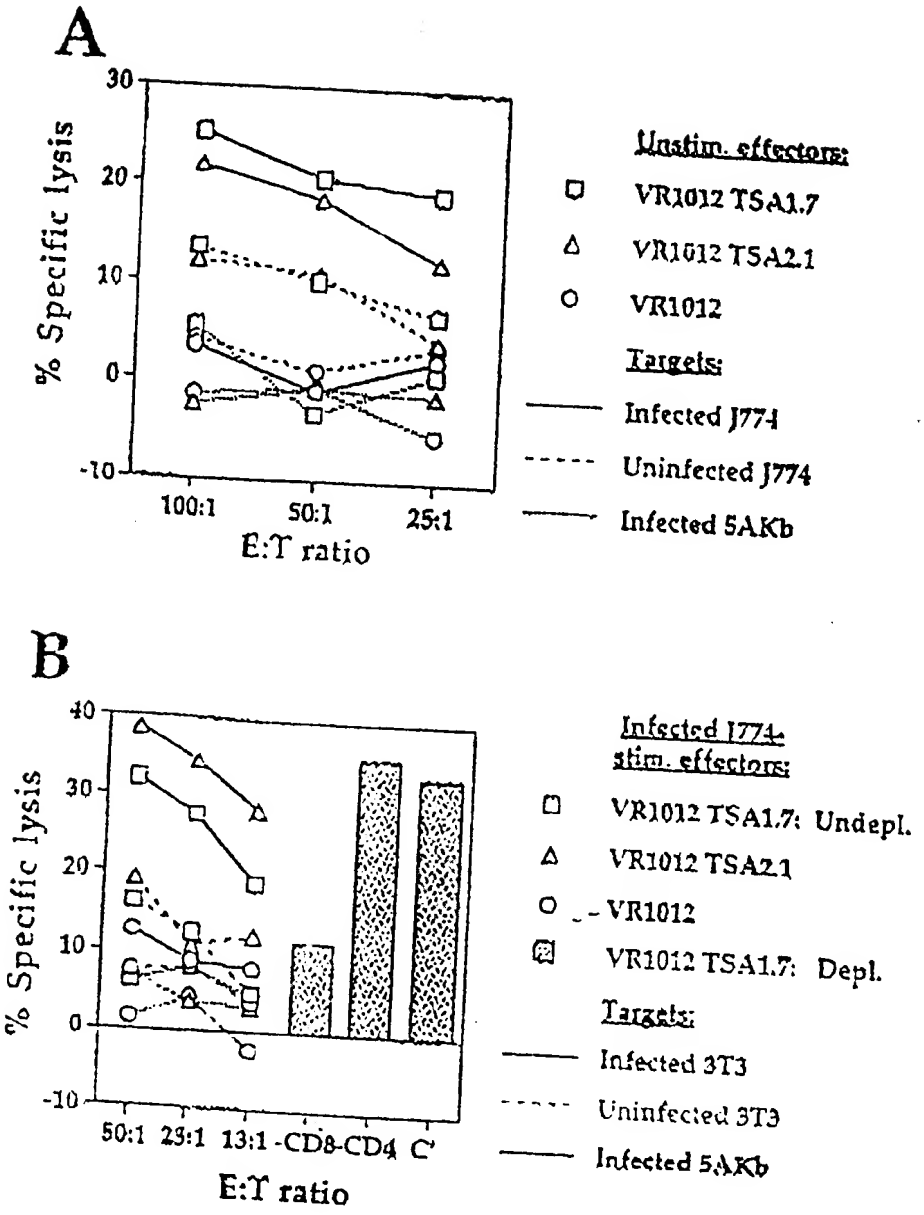

A
% Specific lysis
30
20
10
0
-10
100:1
50:1
25:1
E:T ratio
Unstim. effectors:
VR1012 TSA1.7
VR1012 TSA2.1
VR1012
Targets:
Infected J774
Uninfected J774
Infected 5AKb
B
% Specific lysis
40
30
20
10
0
-10
50:1
25:1
13:1
-CD8
-CD4
C-
E:T ratio
Infected J774-stim. effectors:
VR1012 TSA1.7: Undepl.
VR1012 TSA2.1
VR1012
VR1012 TSA1.7: Depl.
Targets:
Infected 3T3
Uninfected 3T3
Infected 5AKb

Fig. 3

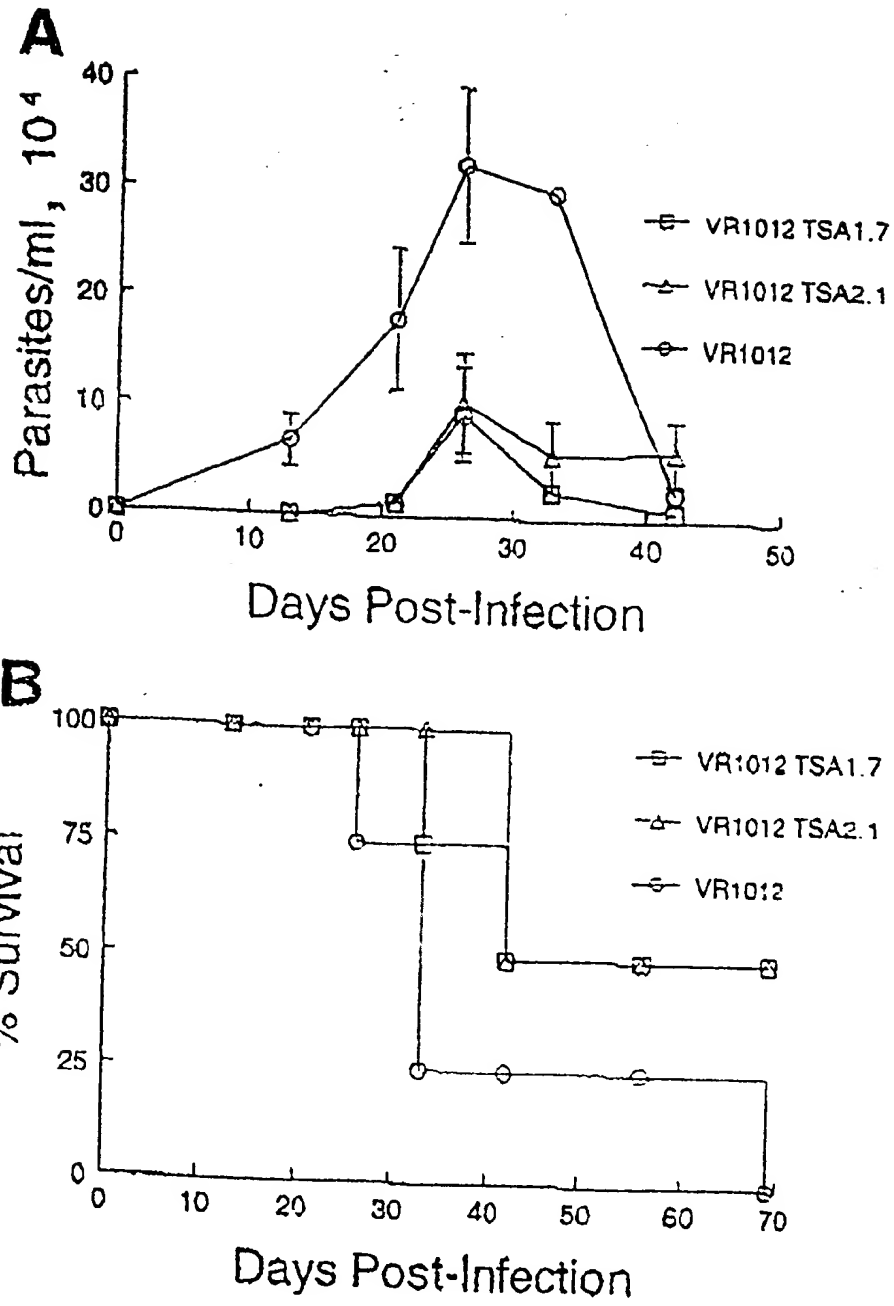

A
Parasites/ml, 10^4
40
30
20
10
0
0
10
20
30
40
50
Days Post-Infection
VR1012 TSA1.7
VR1012 TSA2.1
VR1012
B
% Survival
100
75
50
25
0
0
10
20
30
40
50
60
70
Days Post-Infection
VR1012 TSA1.7
VR1012 TSA2.1
VR1012

Fig. 4

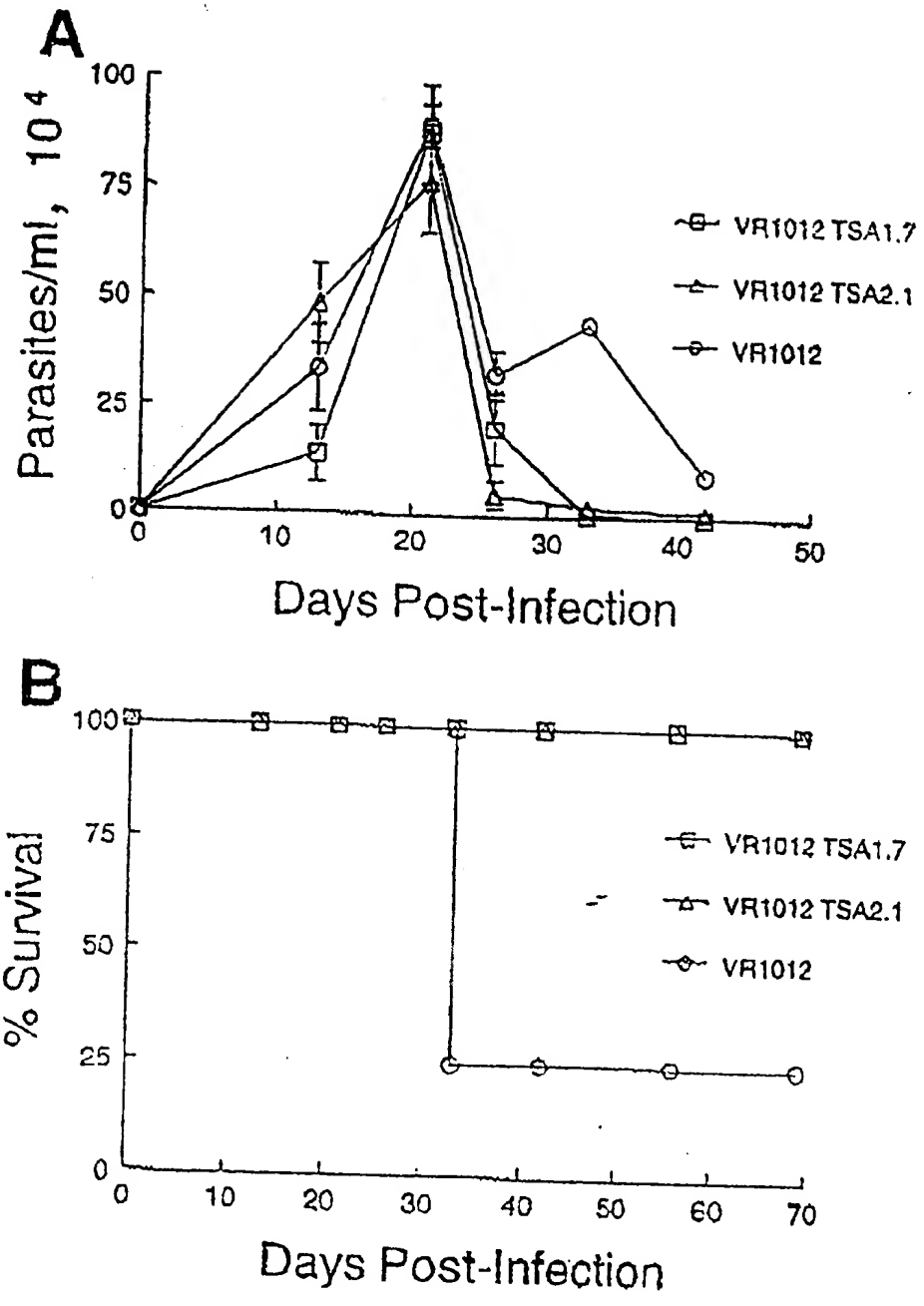

A
Parasites/ml, 10^4
100
75
50
25
0
0
10
20
30
40
50
Days Post-Infection
VR1012 TSA1.7
VR1012 TSA2.1
VR1012
B
% Survival
100
75
50
25
0
0
10
20
30
40
50
60
70
Days Post-Infection
VR1012 TSA1.7
VR1012 TSA2.1
VR1012

Fig. 5

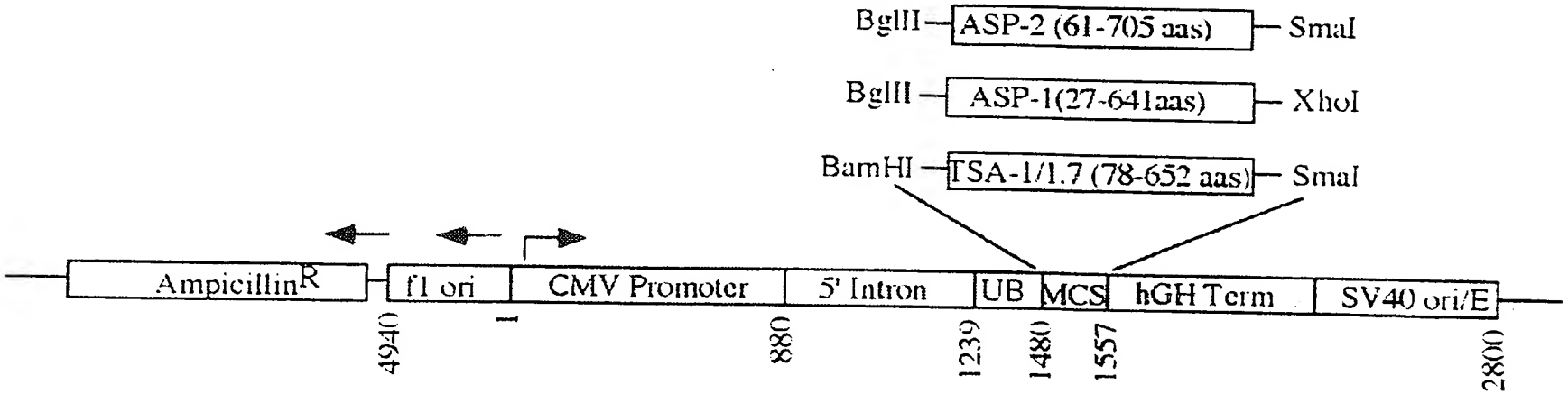
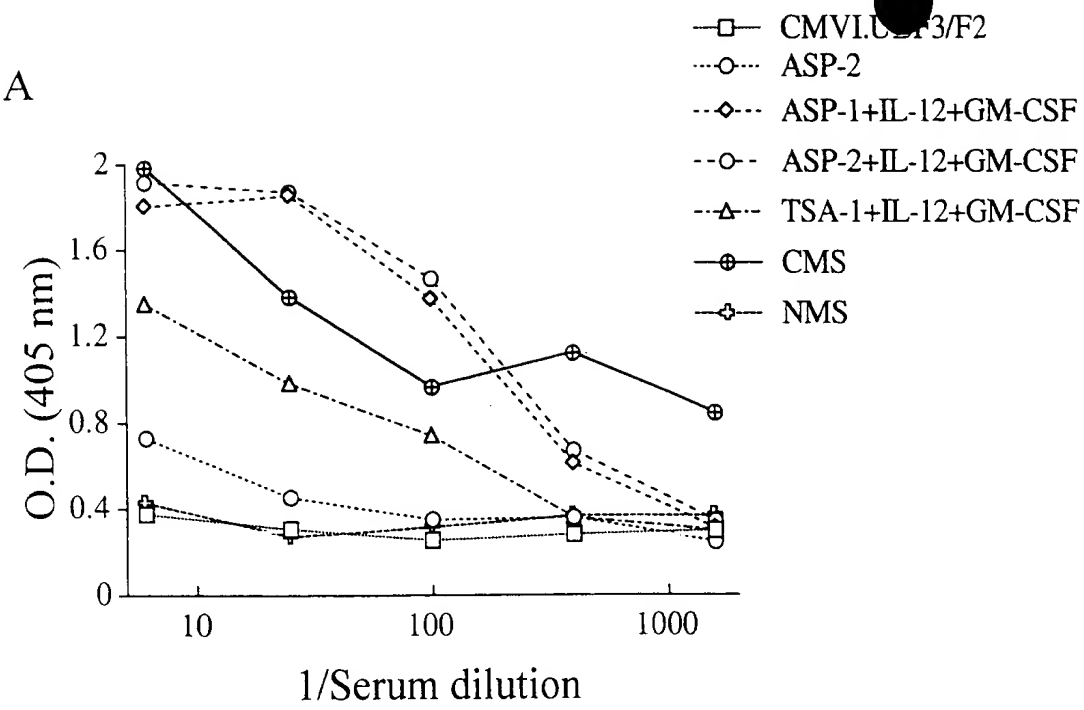

BglII
ASP-2 (61-705 aas)
SmaI
BglII
ASP-1(27-641aas)
XhoI
BamHI
TSA-1/1.7 (78-652 aas)
SmaI
Ampicillin^R
f1 ori
CMV Promoter
5' Intron
UB
MCS
hGH Term
SV40 ori/E
4940
1
880
1239
1480
1557
2800

Fig. 6

A


CMVI.U.F3/F2
ASP-2
ASP-1+IL-12+GM-CSF
ASP-2+IL-12+GM-CSF
TSA-1+IL-12+GM-CSF
CMS
NMS
O.D. (405 nm)
2
1.6
1.2
0.8
0.4
0
10
100
1000
1/Serum dilution

B

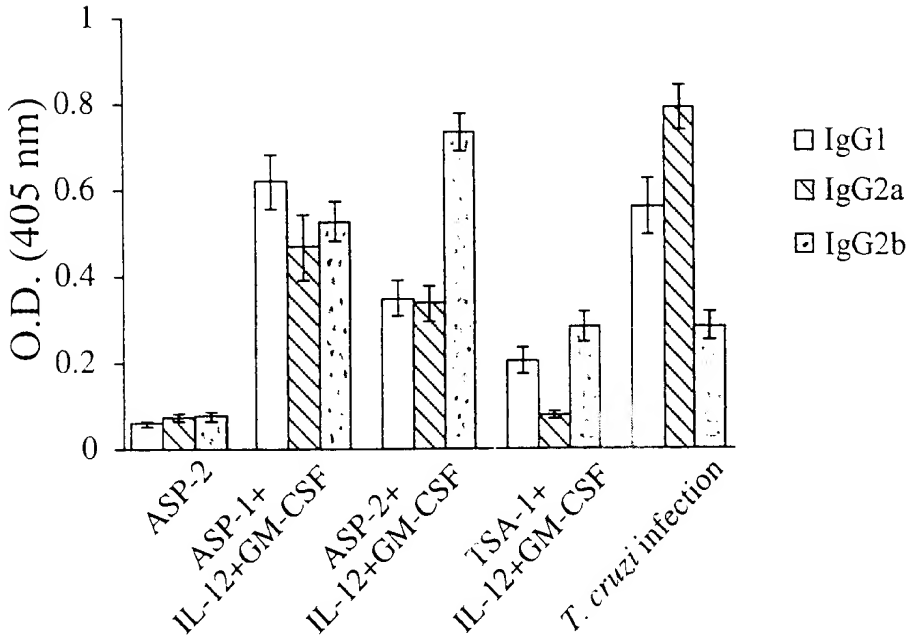
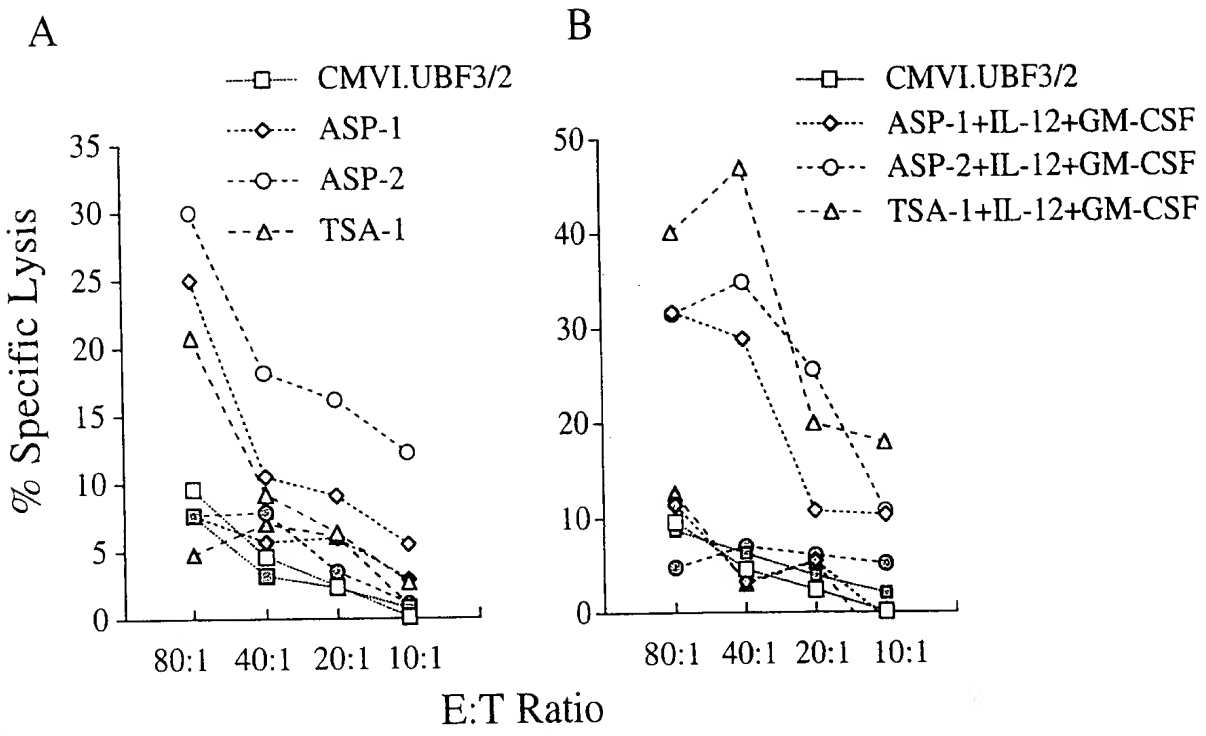

O.D. (405 nm)
1
0.8
0.6
0.4
0.2
0
IgG1
IgG2a
IgG2b
ASP-2
ASP-1+ IL-12+GM-CSF
ASP-2+ IL-12+GM-CSF
TSA-1+ IL-12+GM-CSF
T. cruzi infection

Fig. 7


A
B
CMVI.UBF3/2
ASP-1
ASP-2
TSA-1
ASP-1+IL-12+GM-CSF
ASP-2+IL-12+GM-CSF
TSA-1+IL-12+GM-CSF
% Specific Lysis
0
5
10
15
20
25
30
35
40
50
80:1
40:1
20:1
10:1
E:T Ratio

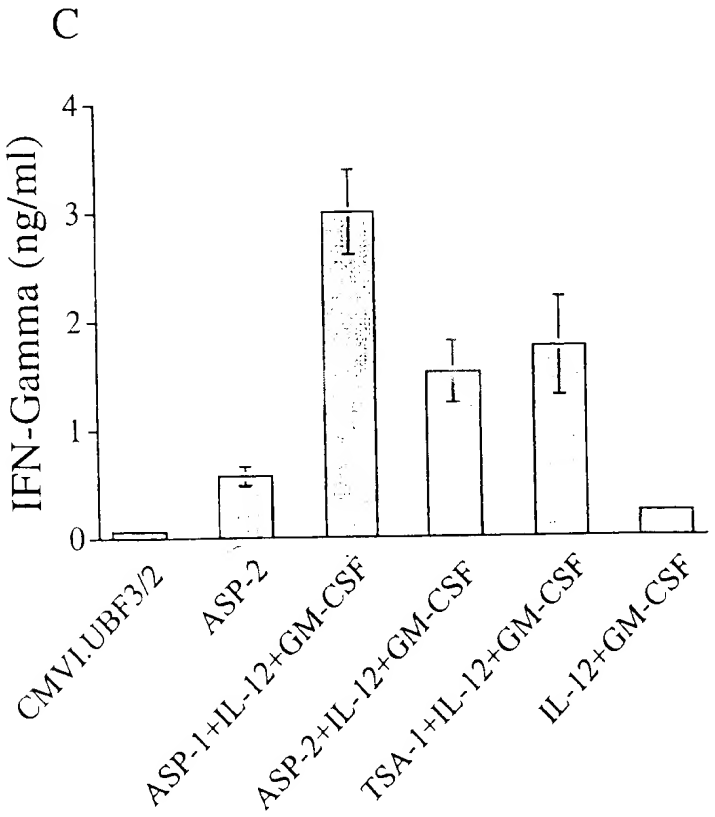

C
IFN-Gamma (ng/ml)
0
1
2
3
4
CMVI.UBF3/2
ASP-2
ASP-1+IL-12+GM-CSF
ASP-2+IL-12+GM-CSF
TSA-1+IL-12+GM-CSF
IL-12+GM-CSF

Fig. 8

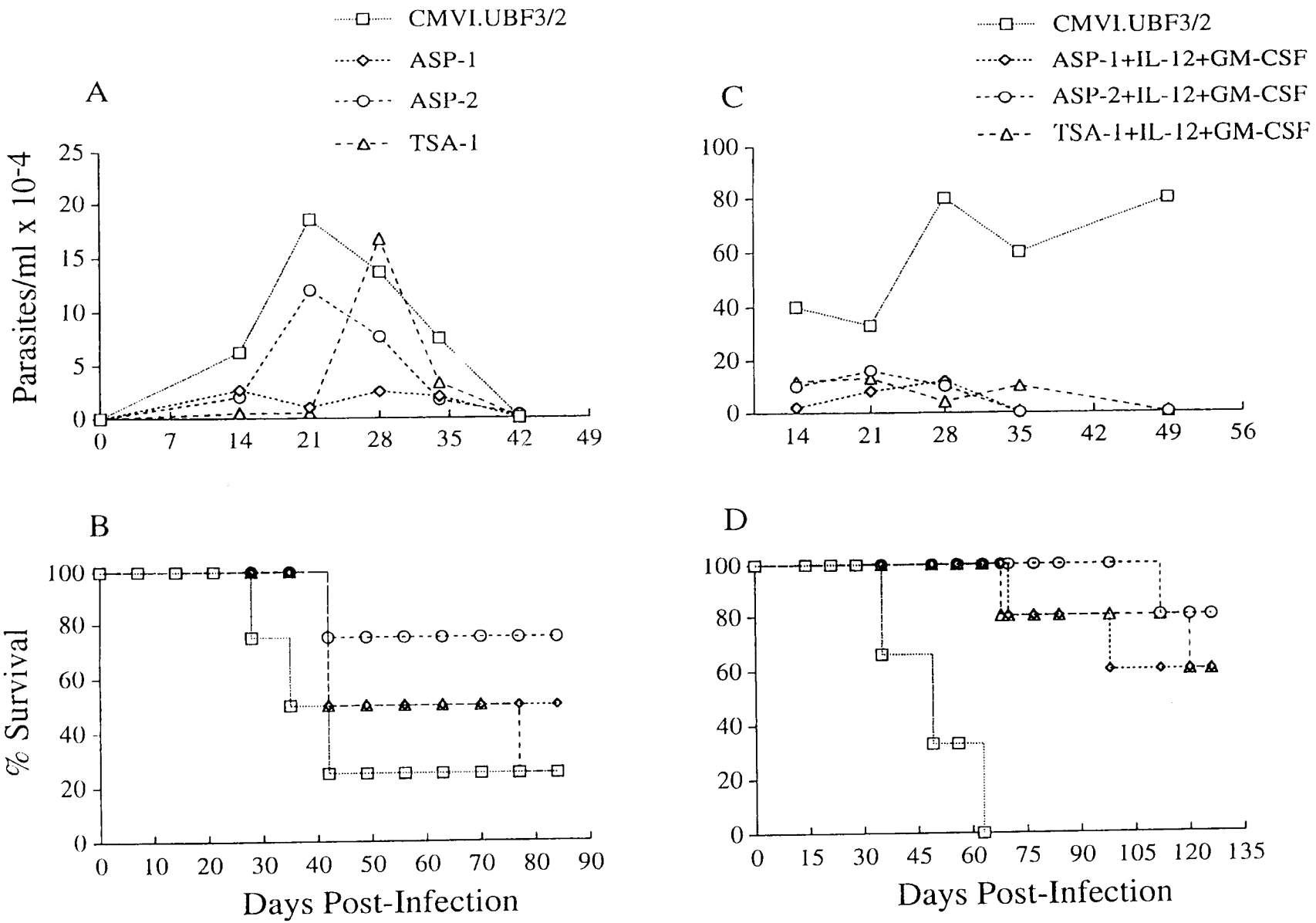

A
CMVI.UBF3/2
ASP-1
ASP-2
TSA-1
Parasites/ml x 10-4
25
20
15
10
5
0
0
7
14
21
28
35
42
49
C
CMVI.UBF3/2
ASP-1+IL-12+GM-CSF
ASP-2+IL-12+GM-CSF
TSA-1+IL-12+GM-CSF
100
80
60
40
20
0
14
21
28
35
42
49
56
B
% Survival
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Days Post-Infection
D
100
80
60
40
20
0
0
15
30
45
60
75
90
105
120
135
Days Post-Infection

Fig. 9

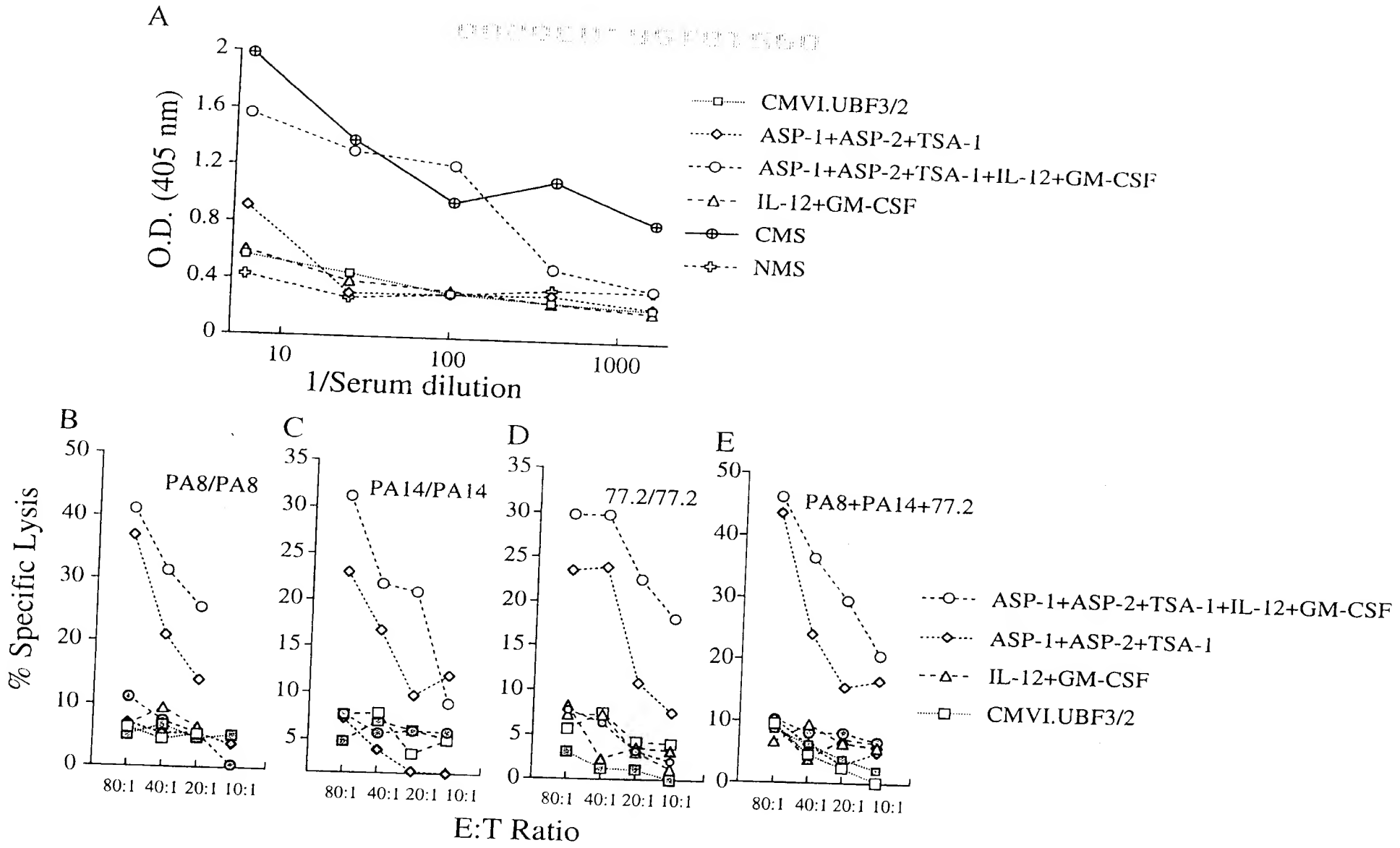

A
O.D. (405 nm)
2
1.6
1.2
0.8
0.4
0
10
100
1000
1/Serum dilution
CMVI.UBF3/2
ASP-1+ASP-2+TSA-1
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
IL-12+GM-CSF
CMS
NMS
B
C
D
E
% Specific Lysis
PA8/PA8
PA14/PA14
77.2/77.2
PA8+PA14+77.2
80:1 40:1 20:1 10:1
E:T Ratio
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
ASP-1+ASP-2+TSA-1
IL-12+GM-CSF
CMVI.UBF3/2

Fig. 10

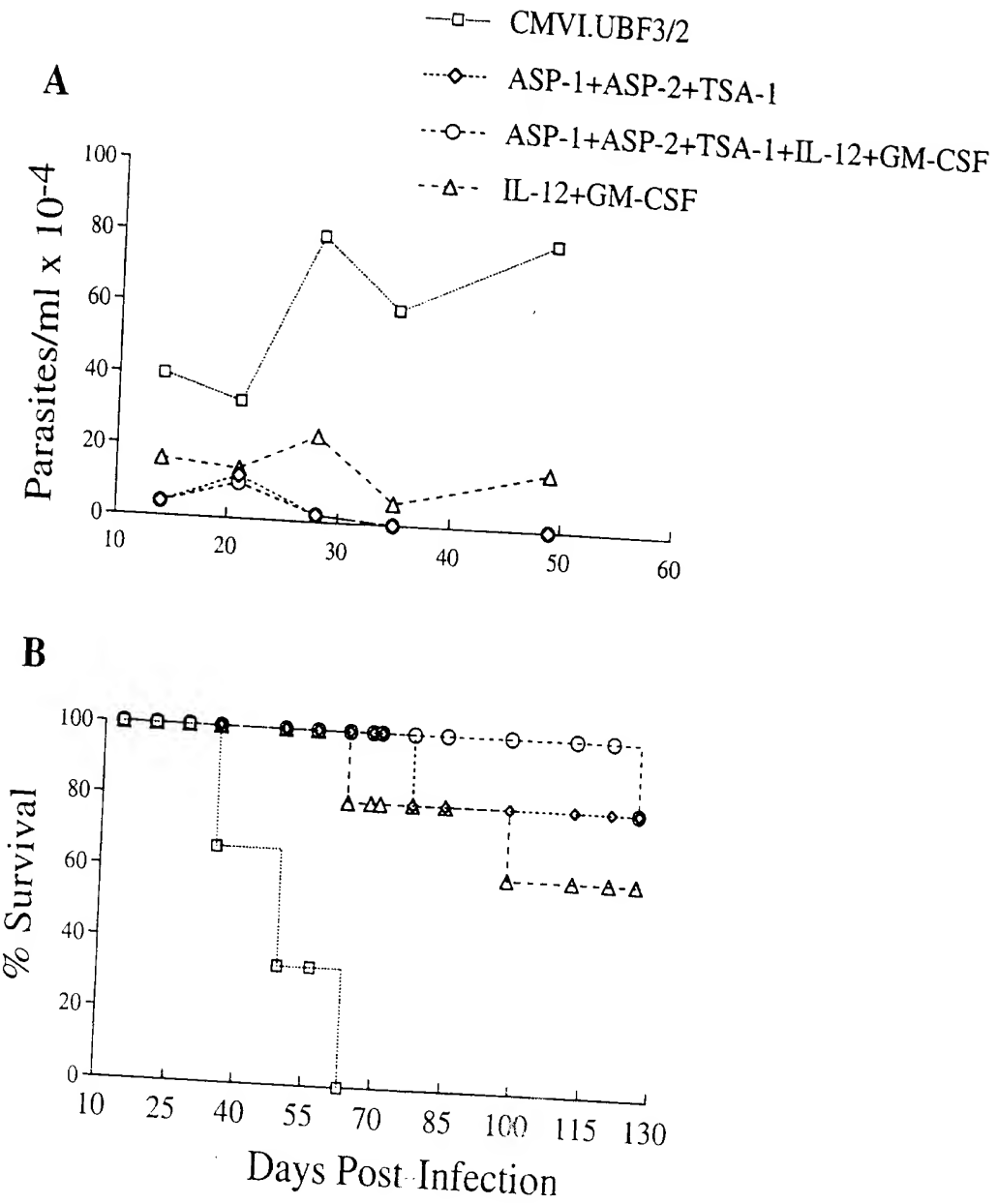

A
CMVI.UBF3/2
ASP-1+ASP-2+TSA-1
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
IL-12+GM-CSF
Parasites/ml x 10-4
100
80
60
40
20
0
10
20
30
40
50
60
B
% Survival
100
80
60
40
20
0
10
25
40
55
70
85
100
115
130
Days Post Infection

Fig. 11

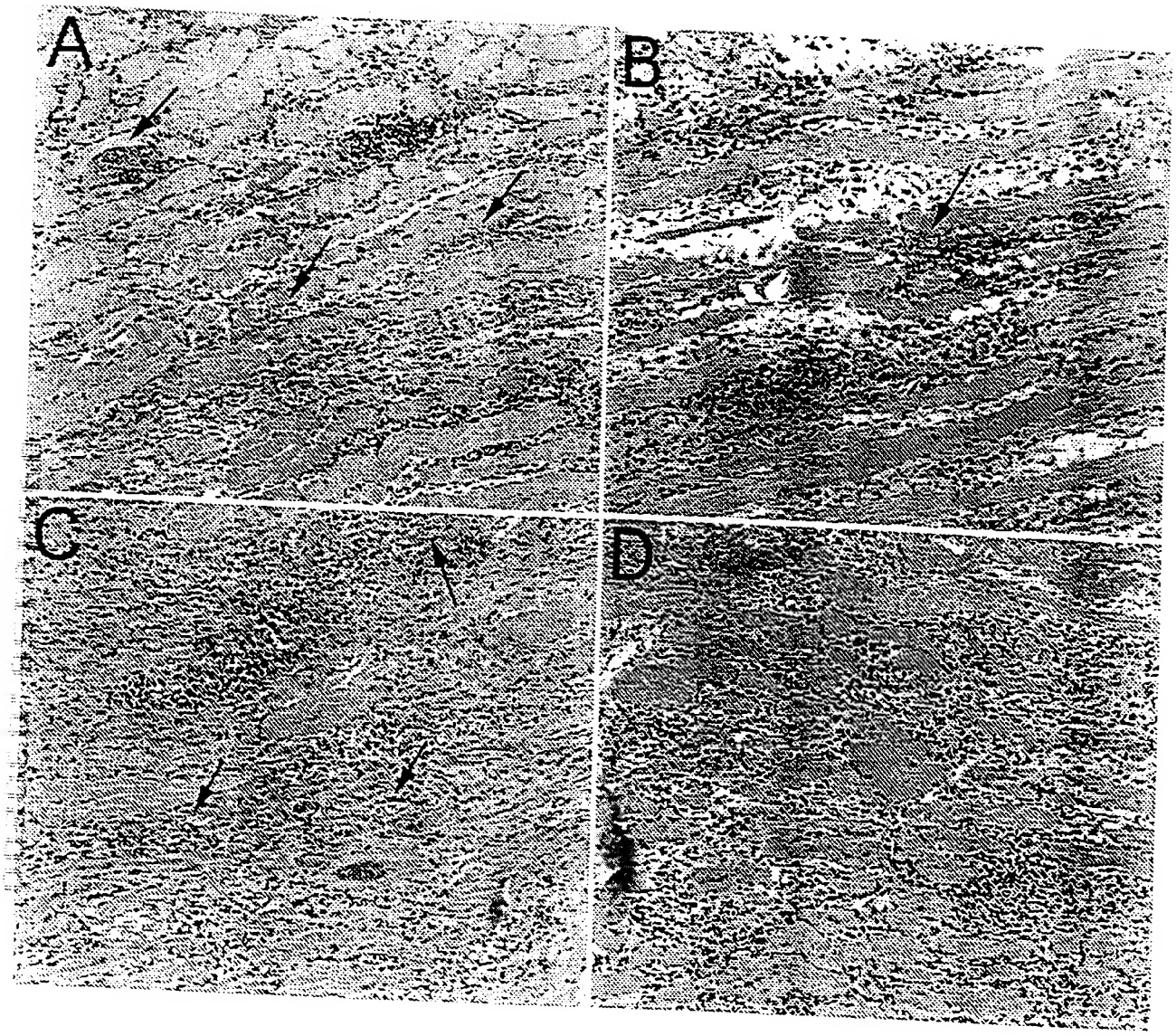


Fig. 12

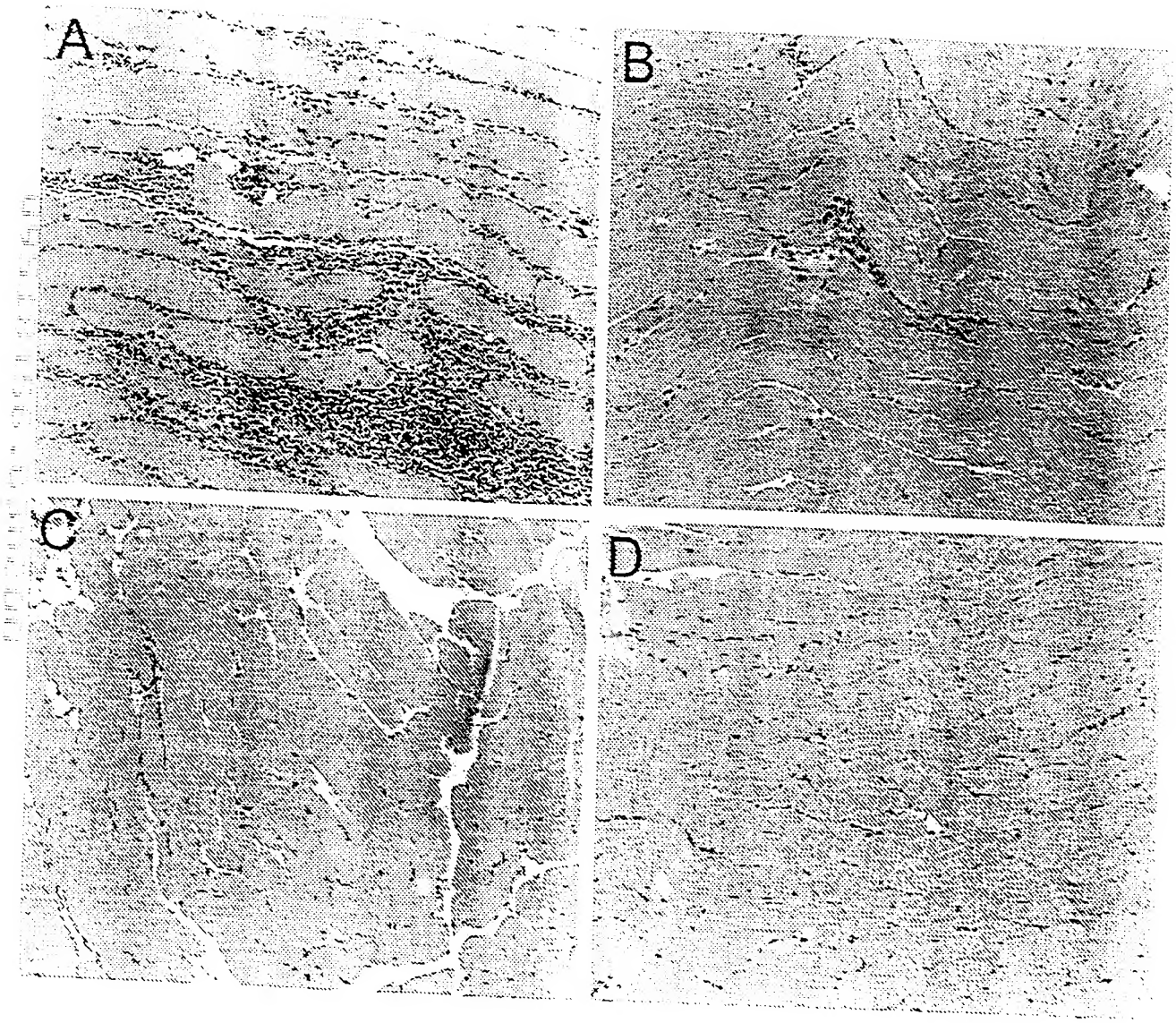


Fig. 13

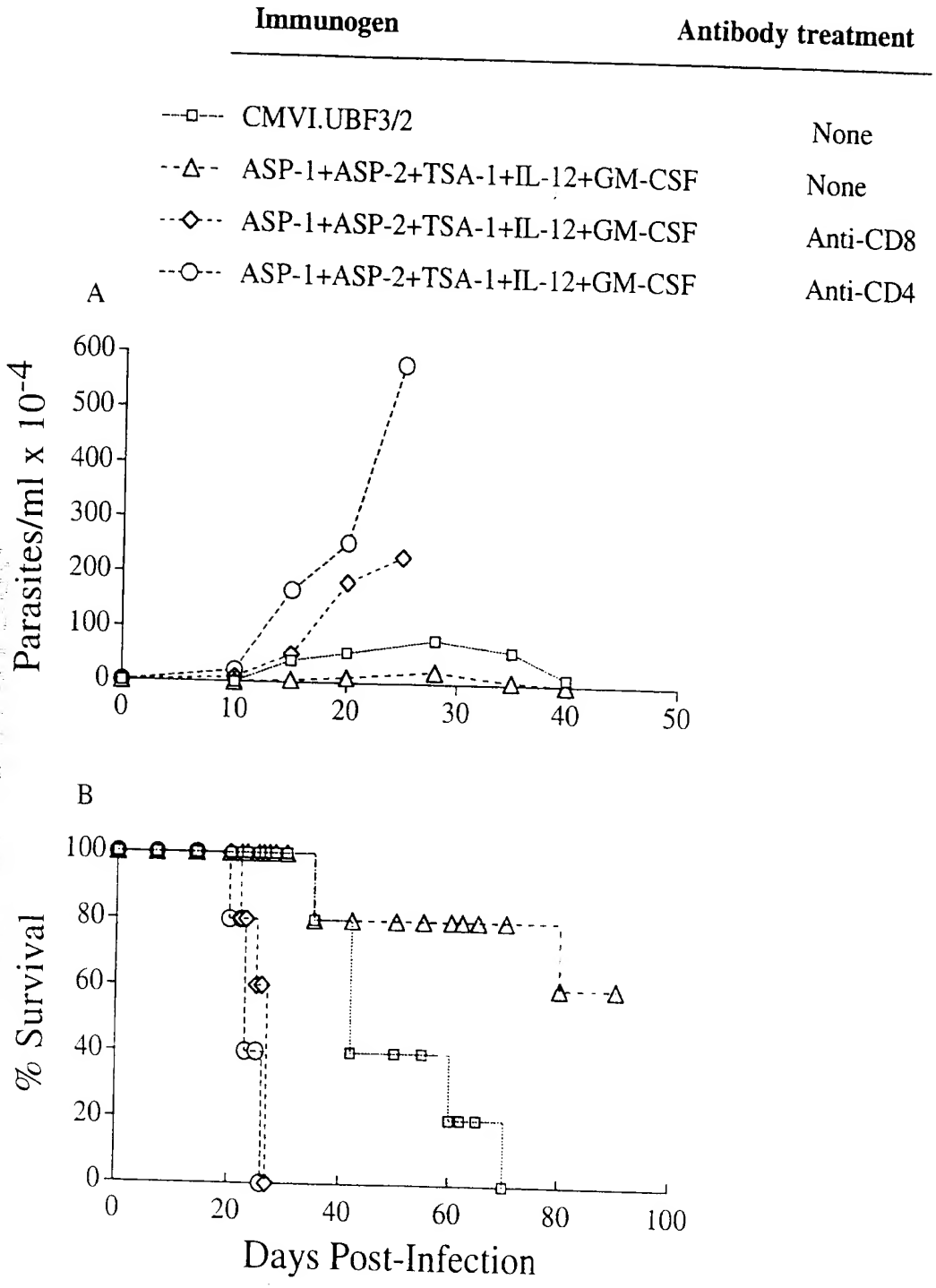

Immunogen
Antibody treatment
CMVI.UBF3/2
None
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
None
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
Anti-CD8
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
Anti-CD4
A
Parasites/ml x 10-4
600
500
400
300
200
100
0
0
10
20
30
40
50
B
% Survival
100
80
60
40
20
0
0
20
40
60
80
100
Days Post-Infection

Fig. 14

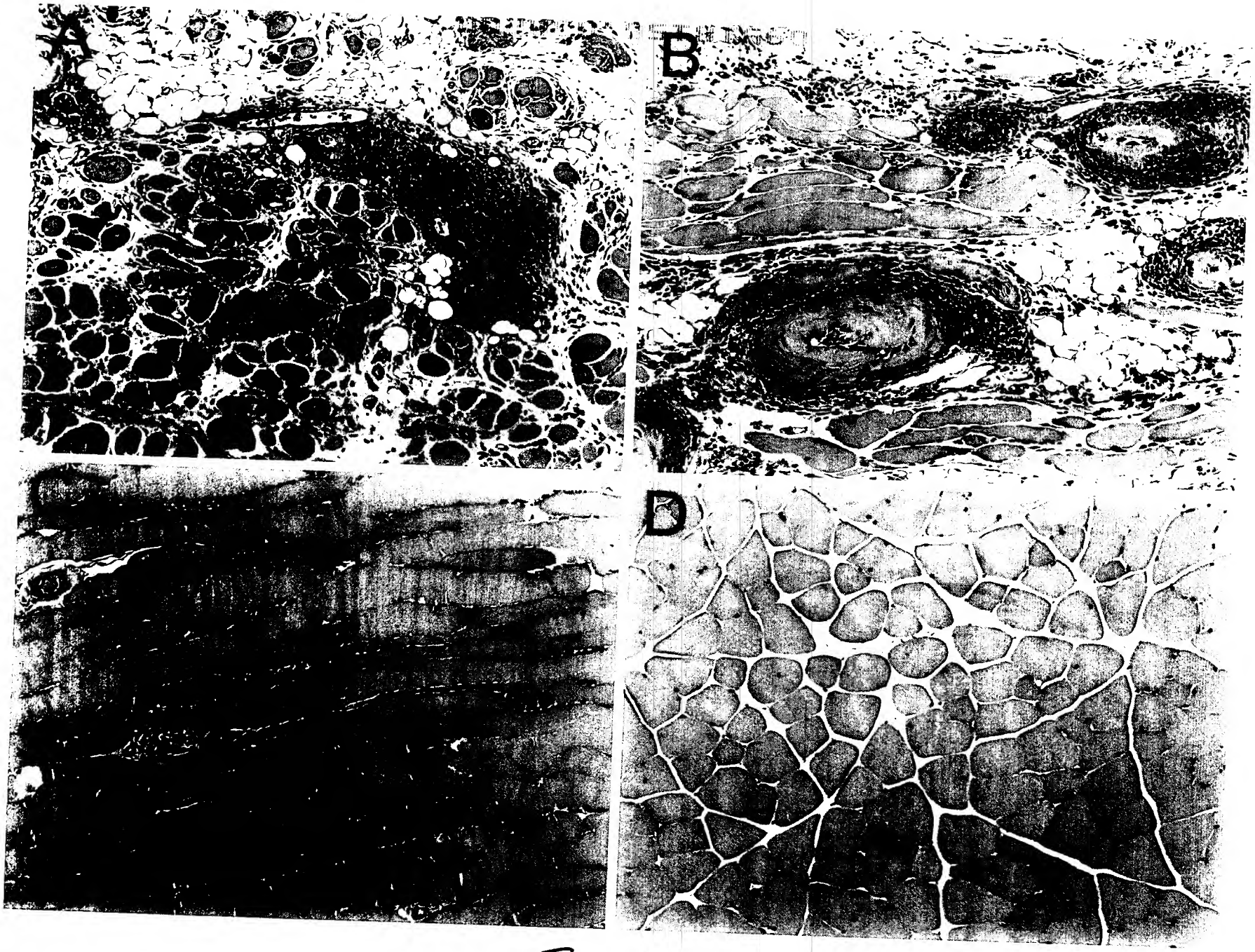


Fig. 15

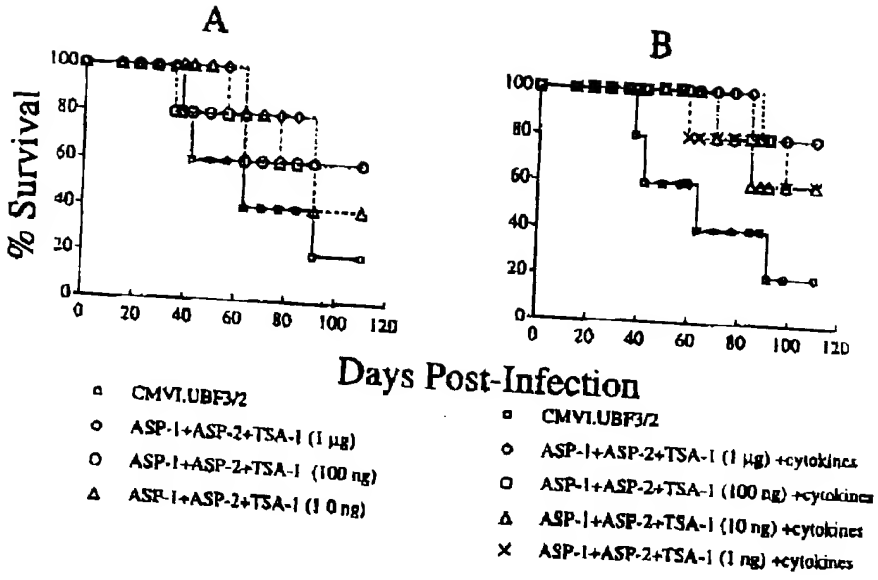

A
B
% Survival
100
80
60
40
20
0
0
20
40
60
80
100
120
Days Post-Infection
CMVI.UBF3/2
ASP-1+ASP-2+TSA-1 (1 µg)
ASP-1+ASP-2+TSA-1 (100 ng)
ASP-1+ASP-2+TSA-1 (10 ng)
CMVI.UBF3/2
ASP-1+ASP-2+TSA-1 (1 µg) +cytokines
ASP-1+ASP-2+TSA-1 (100 ng) +cytokines
ASP-1+ASP-2+TSA-1 (10 ng) +cytokines
ASP-1+ASP-2+TSA-1 (1 ng) +cytokines

Fig. 16

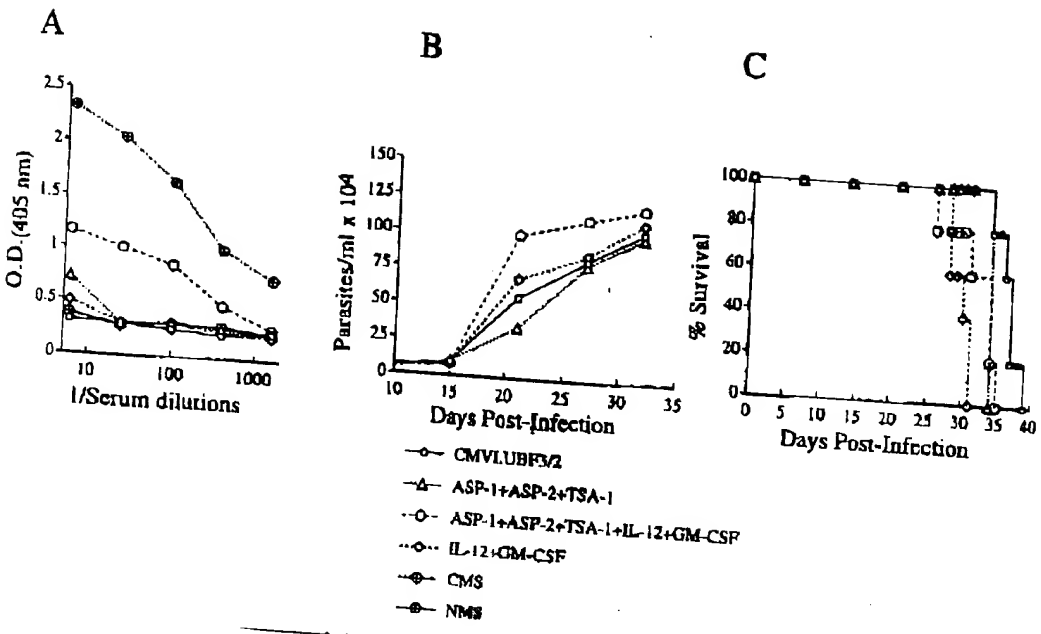

A
B
C
O.D.(405 nm)
1/Serum dilutions
Parasites/ml x 10⁴
Days Post-Infection
% Survival
Days Post-Infection
CMVLUBF3/2
ASP-1+ASP-2+TSA-1
ASP-1+ASP-2+TSA-1+IL-12+GM-CSF
IL-12+GM-CSF
CMS
NMS

Fig. 17

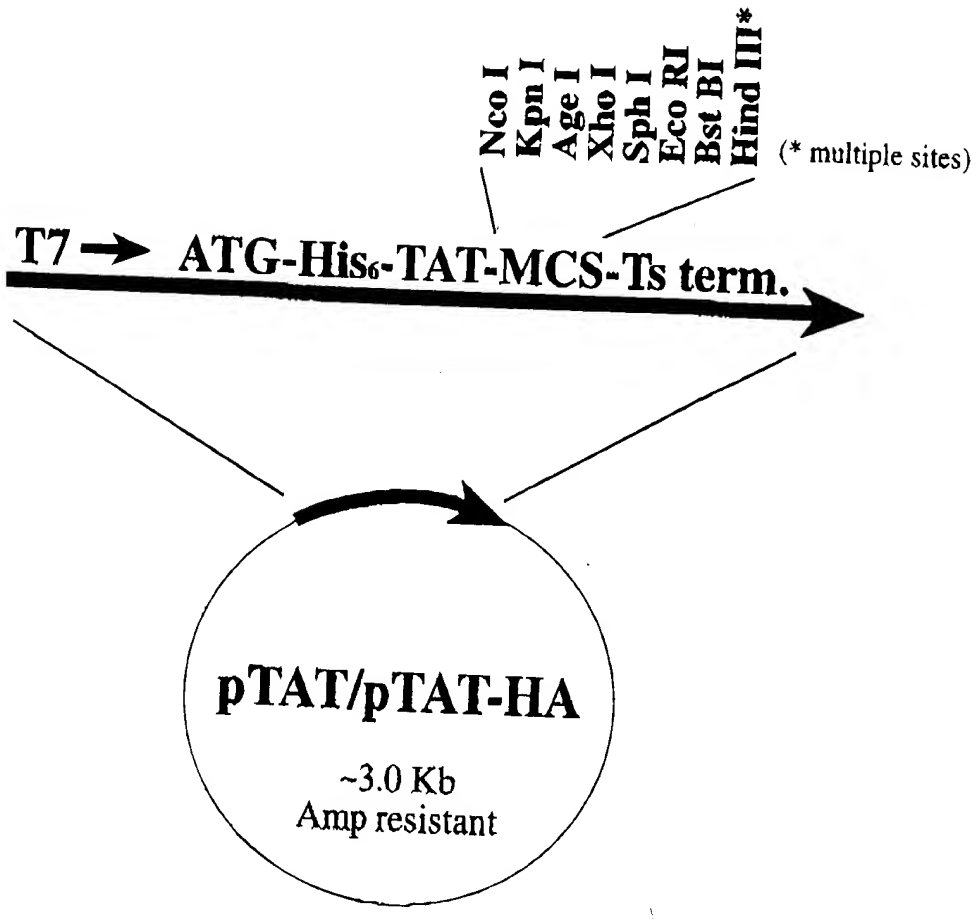

Nco I
Kpn I
Age I
Xho I
Sph I
Eco RI
Bst BI
Hind III*
(* multiple sites)
T7
ATG-His6-TAT-MCS-Ts term.
pTAT/pTAT-HA
~3.0 Kb
Amp resistant

Fig. 18

The porin sequence

ATGCGGAAGAAAGCCGCAGCATTAGCAGCGCCCACAGCAGACACACGGCCGACGTGCCGCGGGGCTGCCATTGCCA
ATAAATTTATGGAACGTGCCGGCCCCCGTGAGGGCGTTGGGAGATCAACGGAGATGCCGGCTGCTGGACCGACGGG
GTCTCAAAGAACTCAAACGCAACGGGAGGTGAAAGCGTCACAAGACGCCGACGCGGCGGCCATTAGTAGTTATTTC
CAGTCCGAATTGGTGACATCTCAGTCGCACGAGGGTGTGTCTCCTCTGGCAAAGACTAGGGCCAACGAACGGCGGA
ACGGGGAGCAGGAGCGGGAGAAGGAACTGCCGGCGGTTGGTGGCGCCGTTCCAACTGGGAAGGGGACGGACCCCAA
ACAGCGAGTGCTGCAGGATTTGCCAGCGATGCACGCGGAGGGACAAAACCAGCACGGTAGAGAGGGTGACAAGGGT
GTTTCCGTGAAGATGGACTCCCCTGGTCGCGTACAGGTGCTGGAGCAAATGTTGCTACACCTGGCTGCATTGAACA
GACAGCTAGAATTAGAACTTATAGAAACGCGACGGGAACTGACGATGTACAAGCAGCTTTTACCTGATGTGCAGCG
CCAGACCGAGGCCCATGCTTTGTCTCAGGAGCATCACAAAGCGAATAGTGCTGCTCCGCCACTGATGTCAGATGAG
AGGCGACGACAGATGCTCTTTACAGGGCAACAACAACAACAGCAACAAGTGGAAGATCTGCATGGCGGTATTAGCG
GGTGGGAAACGGCAGCGAGGAGAATGCGCTATGGTTACGAGGAGGGGGAGAGGGACGCCCTTTCAGATGGTGAGGG
CCGTCCACGTTGCGCAGGTCGTATGGGCTCCCCGAAGAGATTCCTTTCAACACAACCGCCTCGAAGCAGCAGGAAC
CATCGGAACCCTCACGCTGCTAACGGGACAAATGGCAATAGTCATGTTCCCCATTCGTCCAGACAAAAAAGTCACC
CGACAAGAGGAGCTGCTGTAACTTCCGTACCGTTGGCGGCGTCCGCAACCAATCGCCGAGGTCGTTCCATGCGACA
ACATACCCGACCCCGCGGACCTTCTTATCTTTTCGAACGCCTCGACGCTGAGGATGCAATTGATATGCTGGAGACG
CTGAAGCGCTCTCTCATGTATCGCTGCAACCACTCGCATCATCGATCAACAGAAGGAGATGTTGTGCGGCCCGCCG
CGAAGCCCCGGAAAGGCACGCGGTCTGTTCCACCACCACCGCCACCACCGCCCATGTCATCATCGTCACAAAGAAA
GCTTGCCGCCGCAGTTGCTGGAGCGCCGGCATGCAGCGTCTCAGCACGACACGGAAGGAACCATGGCGTTTCTGCG
GTGGGAGATCCGTCAAGGGGCAATCGAGTTTCAGAAACAGCTCGCATAGCTCATGCTCCTTCTTTTGGGGGGAAGA
AATGCGCGCCGGGCCTAACCCAACTCCATTTCTCTTCCCCTTCCAGAAGGGCTACGCCGATGAAAAAAGACACGCC
ATTGTCACGTGGTCAAGCGGCTGGAGTAGCAGCAGTAGCGGTGGGCGGTGACGGGCAGCTAGAGGCACTGCAGAGG
CGTTACTGGGAACAGTCCCGTGCGATATTGGAGCAGCTTGAAAACATGCTGGCAGCTGAT

Fig. 19